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Benthic Distribution of Sewage Sludge Indicated by *Clostridium perfringens* at a Deep-Ocean Dump Site†

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Clostridium perfringens **in sediment samples collected at the Deep Water Municipal Sewage Sludge Disposal Site (also called the 106-Mile Site), off the coast of New Jersey, was enumerated. The counts of *C. perfringens* found in sediment samples collected within and to the southwest of the 106-Mile Site were significantly elevated ($P < 0.01$) compared with counts of samples from reference stations of similar depth (2,400 to 2,700 m), topography, and distance from the continental shelf, indicating that the benthic environment was contaminated by sewage dumping at this site. Low counts of *C. perfringens* in sediment samples collected at stations between the base of the continental shelf and the 106-Mile Site indicated that coastal runoff was not a significant source of contamination. Elevated counts were observed for samples up to 92 km to the southwest, whereas low counts were obtained for samples from stations to the east of the 106-Mile Site. This distribution is consistent with previous model predictions of sludge deposition. In areas heavily impacted by sludge dumping, *C. perfringens* counts were generally highest in the top 1 cm of sediment and exceeded 9,000 CFU g (dry weight) of sediment^{-1}. The patterns of *C. perfringens* dispersal observed in this study have proved useful for selection of heavily impacted areas and control stations for further ecological evaluation by a multidisciplinary research team.**

Since 1986, sewage sludge from New York and northern New Jersey has been dumped 196 km (106 nautical miles) off the coast of New Jersey, at the Deep Water Municipal Sewage Sludge Disposal Site (also called the 106-Mile Site). The wastes are transported to this site by barges and gradually discharged into water, where depths are between 2,340 and 2,740 m (4). Approximately 8×10^6 wet tons (ca. 7.26×10^9 kg [wet weight]) of sewage sludge have been discharged at this site per year since 1988 (15), representing about 50% of the sludge dumped at sea worldwide (16). A multidisciplinary investigation is assessing the fate of this sludge and its impact on the benthic ecosystem; as part of this study, the distribution of sludge contamination of the benthic environment has been determined by using *Clostridium perfringens* as an indicator of sewage sludge.

C. perfringens was chosen as the indicator organism of sewage contamination at this site because it forms highly resistant endospores expected to persist under the extreme conditions of the marine benthic environment. Sediments and benthic water at these depths are typically at temperatures of ca. 2°C and pressures of 250 atm (ca. 25.3 MPa). Coliforms and fecal streptococci, standard indicators of fecal contamination, are not expected to remain culturable under these conditions, although this remains to be proven. The use of *C. perfringens*, which is consistently present in sewage, has previously been proposed as an alternative indicator of fecal contamination under conditions where survival properties of the water quality indicators are critical (3). *C. perfringens* has proved useful in previous studies tracing sewage wastes in the marine environment (6, 17).

It was expected that background levels of *C. perfringens* at this remote marine site would be low, since *C. perfringens* levels in North Sea sediments have been shown to be very low (the highest concentration of clostridia obtained from 10 stations was 4 CFU g [wet weight] of sediment^{-1}) (7). Small numbers of *C. perfringens* spores may be present in ocean sediments as a result of deposition of fecal material by marine birds and atmospheric transport of soil particles (5, 18) and sediment transport from coastal waters (2). *C. perfringens* is present in sewage sludge in numbers several orders of magnitude greater than those in soil or sediment. *C. perfringens* counts of approximately 10^6 spores g^{-1} in sewage sludge and 10^1 to 10^2 spores ml^{-1} in effluents from sewage treatment plants have been reported (9). Therefore, it was expected that contamination of the benthic environment by sludge would result in a readily detectable increase in counts of *C. perfringens*.

The patterns of dispersal and distribution of sludge on the ocean floor arising from dumping at the 106-Mile Site have been the subject of considerable speculation. There have been several attempts to create a model of the dispersal pattern at this site (11–14). A recent model proposed by Fry and Butman (8), using current data obtained near the 106-Mile Site and experimentally determined settling velocities of sewage sludge (10), predicts a maximum flux in the southwestern corner of the site of 60 mg m^{-2} day^{-1}, decreasing to 25 and 1 mg m^{-2} day^{-1} at distances of 50 and 350 km to the southwest, respectively. This model predicts that little material will reach the continental shelf and that fine sludge particles will be widely dispersed in the water column.

Reliable indicators of sewage contamination of the benthic environment are necessary if the predictions of these models are to be validly tested. In an initial study, *C. perfringens* spore counts in sediments from the vicinity of the 106-Mile Site indicated that these sediments were indeed being contaminated by sludge deposition. *C. perfringens* counts cor-

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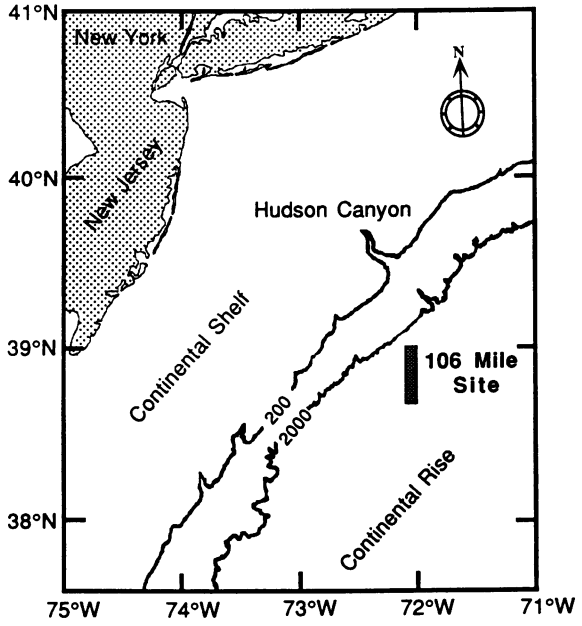

FIG. 1. Location of the Deep Water Municipal Sewage Sludge Disposal Site.

related well with nonmicrobial indicators of sewage contamination, such as silver, linear alkylbenzenes, and coprostanol (4). More extensive sampling and enumeration of *C. perfringens* are reported here, to elucidate the pattern of sludge distribution in the benthic environment and provide additional data for comparison with model predictions.

The dumping of sewage sludge in the marine environment is a controversial method of disposal because of concern over the long-term environmental impact of this course of action. The Ocean Dumping Ban Act of 1988 required sludge dumping at the 106-Mile Site to end by the end of 1991. However, New York City continued dumping until July 1992 and paid penalties for this action. Monitoring of the 106-Mile Site during the period of sludge dumping and in subsequent years provides an opportunity to assess the long-term effects of deep ocean sludge dumping on benthic ecology.

MATERIALS AND METHODS

Sample collection. The location of the 106-Mile Site is shown in Fig. 1, and the locations of sample sites are given in Table 1. Research cruises were accomplished during August and November 1990 on board the R/V *Oceanus* and are designated OC222 and OC227, respectively. Bottom sediment samples were collected by using surface-deployed box corers (0.5 by 0.5 m in cross section) containing 25 subcores (each 0.1 by 0.1 m in cross section). Box corers were designed to retrieve sediment to a depth of ca. 0.5 m. Immediately on retrieval of the sampling apparatus, the overlying water on each of three subcores was carefully removed and the subcores were sampled with 10-ml sterile syringes modified by removal of end flanges to form open cylinders. Syringes were inserted into sediment to a depth of at least 5 cm, to obtain an undisturbed depth profile. Syringes containing samples were sealed, maintained at 4°C, and transported to a land-based laboratory for analysis.

Enumeration of *C. perfringens*. Sediment was extruded from the sampling syringes in 1-cm divisions and diluted in 1% (wt/vol) sterile saline. Divisions of 1 cm were sampled to a total depth of 5 cm and 3 cm for samples from the OC222 and OC227 cruises, respectively. The dry weight of the sediment in each 1-cm division was determined, and the *C. perfringens* count was expressed in CFU per gram of moisture-free sediment. Dilutions were plated on mCP medium, designed for the isolation of *C. perfringens* (3), or on modified mCP medium (1). The media were incubated for 24 h in an anaerobic atmosphere at 45°C before enumeration of *C. perfringens* colonies. Randomly selected isolates were further characterized by using the API 20A anaerobe identification system (API Analytab Products, Plainview, N.J.) to confirm the efficacy of the mCP medium.

TABLE 1. Locations of sampling stations on cruises OC222 and OC227 and mean log *C. perfringens* counts in the upper 1 cm of sediment at each station

Cruise and station	Location		Mean[a] log *C. perfringens* CFU g^{-1} ± SD
	Latitude west	Longitude north	
OC222			
1	39° 18.83′	71° 12.38′	1.72 ± 0.059
2	39° 15.16′	71° 30.07′	<1.64
3	38° 41.17′	72° 06.78′	3.71 ± 0.171
4	38° 56.92′	72° 05.43′	3.58 ± 0.065
5-1	38° 54.56′	72° 07.39′	3.56 ± 0.136
5-2	38° 54.58′	72° 07.39′	3.76 ± 0.186
5-3	38° 54.52′	72° 07.38′	3.56 ± 0.408
7-1	38° 54.43′	72° 05.40′	3.47 ± 0.176
7-2	38° 54.44′	72° 05.38′	3.50 ± 0.239
7-3	38° 54.45′	72° 05.43′	1.91 ± 0.319
8	38° 59.71′	72° 10.20′	3.26 ± 0.232
9	39° 03.94′	72° 02.95′	2.62 ± 0.119
10	38° 49.16′	72° 06.70′	3.96 ± 0.071
11	38° 49.29′	72° 02.77′	3.77 ± 0.209
OC227			
14	38° 47.46′	72° 05.06′	3.52 ± 0.207
15	38° 48.13′	71° 54.31′	3.09 ± 0.287
16	38° 34.19′	72° 29.52′	3.55 ± 0.155
17	39° 15.09′	71° 10.95′	1.95 ± 0.546
18	38° 53.49′	71° 28.82′	2.43 ± 0.741
19	38° 49.92′	71° 40.80′	2.55 ± 0.371
20	38° 49.27′	71° 59.97′	2.77 ± 0.258
21-1	38° 16.03′	72° 52.62′	2.97 ± 0.308
21-2	38° 16.03′	72° 52.60′	2.66 ± 0.388
21-3	38° 16.04′	72° 52.63′	2.70 ± 0.540

[a] Means of three subcores per box corer except for data from stations 14 and 21-1, which are means of two subcores.

Sampling strategy and statistical analyses. Two or three plate counts were done for each subcore sample, and three subcores were sampled for each box corer sample. In three cases (stations OC222-5, OC222-7, and OC227-21), three box corer samples were analyzed.

After it was determined that the data were normally distributed, analysis of variance was conducted on log-transformed data by using a general linear models procedure in the statistics analysis system (SAS) on the James Madison University VAX mainframe computer. The means and variance estimates were also generated with the SAS program.

RESULTS

Confirmation of presumptive *C. perfringens* colonies. Presumptive *C. perfringens* colonies were selected at random and identified by using the API 20A anaerobe identification

TABLE 2. Identification and biochemical characteristics of random isolates from mCP medium designated as *C. perfringens* by their characteristic reactions on this medium

Identification	No. of isolates	Results on API 20A test substrate[a]:																				
		IND	URE	GLU	MNS	LAC	SAC	MLT	SAL	XYL	ARA	GEL	ESC	GLY	CEL	MNE	MLZ	RAF	SOR	RHA	TRE	CAT
C. perfringens[b]	2	−	−	+	−	+	+	+	−	−	−	+	−	+	−	+	−	−	−	−	−	−
C. perfringens[b]	8	−	−	+	−	+	+	+	−	−	−	+	−	+	−	+	−	−	−	−	+	−
C. perfringens[b]	19	−	−	+	−	+	+	+	−	−	−	+	−	−	−	+	−	−	−	−	+	−
C. perfringens[b]	2	−	−	+	−	+	+	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−
C. perfringens[b]	1	−	−	+	−	+	+	+	−	−	−	+	−	−	−	+	−	−	+	−	+	−
C. perfringens[c]	2	−	−	+	−	+	+	+	−	−	−	+	−	−	−	+	−	+	−	−	−	−
C. perfringens[c]	1	−	−	+	−	+	+	+	−	−	−	−	−	−	−	+	−	−	−	−	+	−
C. perfringens[c]	3	−	−	+	−	+	+	+	−	−	−	+	−	−	−	+	−	+	−	−	+	−
C. perfringens[c]	1	−	−	+	−	−	+	+	−	−	−	+	−	+	−	+	−	−	−	−	−	−
C. perfringens[d]	1	−	−	+	−	−	+	+	−	−	−	+	−	−	−	+	−	−	−	−	+	−
C. perfringens[d]	1	−	−	+	+	+	+	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−
C. perfringens[d]	1	−	−	+	−	−	+	+	+	−	−	−	+	+	+	+	−	−	−	−	−	−
Unknown[e]	1	−	−	+	−	+	+	+	+	−	−	+	+	−	−	+	−	−	−	−	+	−

[a] Abbreviations: IND, indole; URE, urea; GLU, glucose; MNL, mannitol; LAC, lactose; SAC, saccharose; MLT, maltose; SAL, salicin; XYL, xylose; ARA, arabinose; GEL, gelatin; ESC, esculin; GLY, glycerol; CEL, cellobiose; MNE, mannose; MLZ, melezitose; RAF, raffinose; SOR, sorbitol; RHA, rhamose; TRE, trehalose.

[b] API profile index indicates *C. perfringens*, excellent or very good identification.

[c] API profile index indicates *C. perfringens* as first choice, good likelihood but low selectivity.

[d] API computer data base indicates *C. perfringens* as first or second choice, good likelihood but low selectivity.

[e] API computer data base gives unacceptable identification, *C. perfringens* not listed as a possibility.

system. All isolates were found to be anaerobic, gram-positive bacilli. Of 46 colonies selected for further analysis, 42 were confirmed to be *C. perfringens* and 3 were concluded to be presumptive *C. perfringens*. One strain was not identified by the API 20A system. The biochemical profiles of these isolates are given in Table 2. In view of this high confirmation rate, all isolates that were positive on mCP medium or modified mCP medium were counted as *C. perfringens* for the purposes of this study.

Distribution of *C. perfringens* in top sediment. Mean *C. perfringens* counts obtained from the top 1 cm of sediment collected on cruises OC222 and OC227 are shown in Fig. 2 and 3, respectively, and standard deviations of the means are reported in Table 1. Counts are means of triplicate plate counts, except for OC227-14, for which the count is the mean of duplicate plate counts, and OC222-5, OC222-7, and OC227-21, for which triplicate plate counts from two, two, and three independent samples, respectively, have been averaged.

Sampling on cruise OC222 was concentrated within and up to 5 km to the west of the 106-Mile Site, to the northwest of the site (i.e., between the site and the continental shelf), and at control stations to the northeast of the Hudson canyon, ca. 70 km from the site. *C. perfringens* counts from cruise OC222 are shown in Fig. 2. Counts in sediments from stations within and up to 5 km west of the dump site were significantly higher ($P < 0.01$) than those in sediments from stations of similar depth and proximity to the continental shelf and to the east of the 106-Mile Site. *C. perfringens* counts from stations within and up to 5 km west of the dump site were also significantly higher ($P < 0.01$) than counts from sediments collected from stations between the 106-Mile Site and the continental shelf, indicating that shelf runoff was not the primary source of contamination.

Sampling on cruise OC227 was aimed primarily at ascertaining the distance to the southwest for which elevated counts of *C. perfringens* could be detected; counts from this cruise are shown in Fig. 3. Station OC227-21, which is approximately 93 km (50 nautical miles) to the southwest of the 106-Mile Site, has a significantly ($P < 0.05$) elevated *C. perfringens* count relative to those of the three stations furthest to the east (OC227-17, OC227-18, and OC227-19).

Distribution of *C. perfringens* with depth. *C. perfringens*

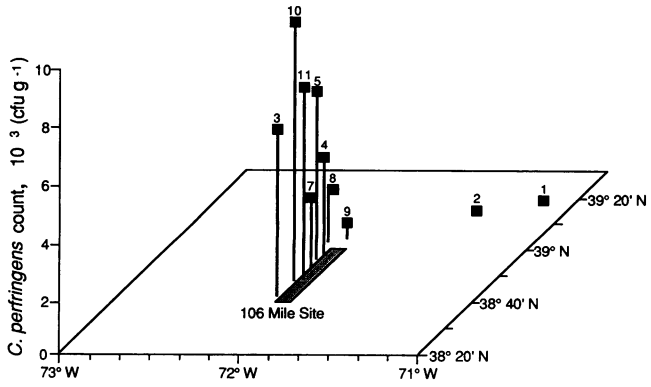

FIG. 2. *C. perfringens* counts obtained from samples collected during cruise OC222. Counts are from the top 1 cm of sediment, in CFU per gram (dry weight) of sediment.

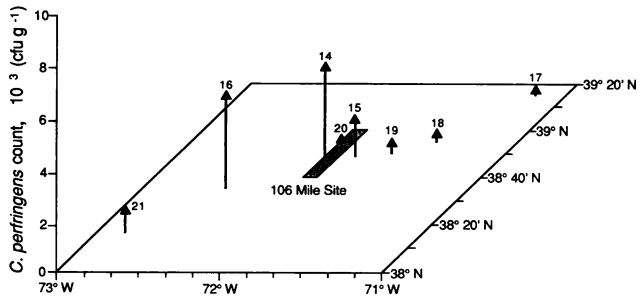

FIG. 3. *C. perfringens* counts obtained from samples collected during cruise OC227. Counts are from the top 1 cm of sediment, in CFU per gram (dry weight) of sediment.

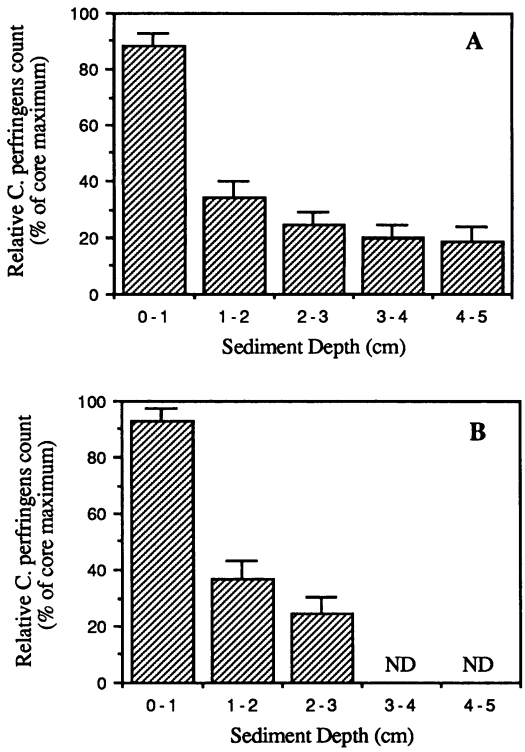

FIG. 4. Distribution of *C. perfringens* at incremental 1-cm depths below the sediment surface for samples from cruises OC222 (A) and OC227 (B). The depth with the highest *C. perfringens* count was set to 100%, and the values for the other four depths were converted to percentages of the maximum.

counts in general showed a strong negative correlation with depth. In 51 samples in which spore counts were greater than 500 spores g (dry weight)$^{-1}$, in at least one of the depth divisions the highest spore count was recorded in the top 1 cm of sediment in 41 cases; i.e., ca. 80% of samples showed the highest *C. perfringens* spore count in the top 1 cm of sediment. The average *C. perfringens* counts by depth, as percentages of the highest count in each sample, for cruises OC222 and OC227 are shown in Fig. 4A and B, respectively.

Sources of variation in *C. perfringens* counts. Multiple samples were obtained at stations 5 and 7 on cruise OC222 and at station 21 on cruise OC227. These samples provide a means of assessing the contribution of sources of variation in *C. perfringens* counts obtained during this investigation. The relative contributions of subcore variations within box corers and box corer variations within stations are shown in Table 3.

TABLE 3. Sources of variation in *C. perfringens* counts at stations where multiple box corer samples were obtained

Site	Mean *C. perfringens* CFU ml^{-1} (10^2) ± SD	% Contribution to sums of squares by source	
		Subcore	Core
OC222-5	68.0 ± 51.45	91.5	8.5
OC222-7	33.9 ± 23.65	89.8	10.2
OC227-21	8.1 ± 7.18	88.1	11.9

DISCUSSION

An understanding of the environmental impact of deep ocean sewage disposal on the benthic environment depends on knowledge of dispersal patterns of sewage after dumping. The counts of *C. perfringens* reported in this study confirm the report of Bothner et al. (4) that sewage sludge is reaching the ocean floor in the vicinity of the 106-Mile Site as a result of sludge dumping at this site. *C. perfringens* counts within the dump site and to the south and west of the dump site were considerably elevated compared with counts to the east of the dump site. Sampling stations to the east of the dump site were similar to those to the west and south in topography and water depth and were located both to the north and south of the Hudson canyon, the prominent topographic feature in this area. *C. perfringens* counts from stations to the east of the 106-Mile Site were consistently below 500 CFU g (dry weight) of sediment^{-1}. *C. perfringens* counts within and to the southwest of the 106-Mile Site were, in contrast, considerably elevated above the background levels recorded to the east of the 106-Mile Site, in some cases by as much as 100-fold. The possibility that these elevated counts result from coastal sources of sewage pollution can be discounted, because sites to the east are at comparable distances offshore and would be similarly impacted by coastal sewage. In addition, sediment samples collected at stations OC222-8 and OC222-9 contained much lower numbers of *C. perfringens* than did those from the heavily impacted stations (Fig. 2). Since OC222-8 and OC222-9 are situated directly between the coast and the 106-Mile Site, at the base of the continental shelf, they would be expected to be more heavily impacted by coastal sewage sources than would stations within and to the southwest of the 106-Mile Site. It can therefore be concluded that the elevated counts of *C. perfringens* in sediments within and to the southwest of the 106-Mile Site are indicative of contamination of the benthic environment by sewage dumped at this site.

The distribution pattern of *C. perfringens* is broadly consistent with the estimates of the sea floor area impacted by sewage sludge in the most recent computer model proposed by Fry and Butman (8) to predict sewage dispersal at the 106-Mile Site. However, our data do indicate that the area of maximum deposition of sludge may be slightly further north than predicted, i.e., toward the middle of and slightly west of the 106-Mile Site, rather than in the southwestern quarter of the site. In addition, it should be noted that no stations to the southeast of the 106-Mile Site have been sampled. The model of Fry and Butman (8) and earlier models (11–14) predicted negligible settling of sewage to the southeast of the site, and it would be interesting to confirm this by ascertaining *C. perfringens* counts in sediments from this area; this will be done in future studies.

The enumeration of *C. perfringens* with the mCP medium of Bisson and Cabelli (3) and modified mCP (1) has proven to be an efficient and reliable method for tracing sewage contamination of deep ocean sediments. This medium provides a convenient method for the enumeration of *C. perfringens* with an acceptable degree of accuracy (Table 2).

The sources of variation in *C. perfringens* counts at stations where multiple samples were obtained are shown in Table 3. The largest source of variation is in counts from subcores taken from the same box corer sample. Variation in counts from multiple box corer samples taken from the same station is much lower. It seems likely that this is indicative of small-scale patchiness in the distribution of *C. perfringens* within the sampling area of 0.25 m^2. This may reflect uneven

settling of sludge particles on the ocean floor or may be the result of local redistribution events, such as resuspension or bioturbation, after the particles reach the bottom.

The results of analysis of sources of variation in *C. perfringens* counts indicate that variation can be reduced by maximizing the number of subcores that are counted within a single box corer sample and illustrates that extensive multiple sampling at a single station is not necessary. Since deployment and retrieval of box corer samplers is time consuming and technically demanding, cruise time and resources can be most efficiently used by maximizing the number of subsamples taken from each sample.

C. perfringens counts have proven to be useful in the context of the multidisciplinary study that is in progress, of which this study is a part, to assess the environmental impact of sewage disposal at the 106-Mile Site. The data reported here, in addition to demonstrating conclusively that sewage is accumulating in the benthic environment and elucidating the pattern of this accumulation, have been valuable in the selection of heavily impacted areas and unimpacted control sites for ecological monitoring. In the absence of fresh inputs of sludge, after cessation of dumping, changes in the distribution of *C. perfringens* spores on the sediment surface will provide important information about resuspension and bioturbation events. The present study has provided valuable baseline data, which will facilitate future comparisons and provide better understanding of the environmental impact of ocean dumping.

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